MICROBIAL SAFETY OF "SALSICCIA SARDA", A TYPICAL ITALIAN SEMI-DRY NATURALLY FERMENTED SAUSAGE

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Keywords: dry fermented sausage, meat processing plants, food microbiology

Introduction

Dry fermented sausages are ready-to-eat products, whose safety is essentially gained by a "hurdle technology" concept (Barbuti and Parolari, 2002). The main hurdles used during the processing of dry fermented products are nitrite and salt content, the decrease of redox potential and water activity (a_w), which inhibit many aerobic bacteria in the early stages of production (*Pseudomonas* spp. and other Gram-negative bacteria), and select the Lactic acid bacteria which cause the pH to fall. These hurdles are essential for the microbial safety and stability of quick-ripened fermented sausages, which are not fully dried. Although dry and semi-dry sausages have rarely been implicated in food poisoning, greater risk should be linked to the consumption of traditional products, because of an empirical application of hurdle technology (Leistner and Gould, 2002). "Salsiccia Sarda" is a typical Italian semi-dry sausage (a_w ranging from 0.90 to 0.95), naturally fermented, and included in the National list of the traditional products (D.M.18/07/2000). The physicochemical and microbiological profiles are unhomogeneous. The presence of spoilage bacteria and hygiene indicators, are strongly linked to the length of ripening, and at times to an incorrect acidification and drying (Mazzette *et al.,* 1998; Greco *et al.,* 2005). The aim of the present study was to evaluate the microbial safety of the various types of "Salsiccia Sarda".

Materials and Methods

Fifty-five samples from different types of ripened "Salsiccia Sarda" were collected from twenty-two processing plants, with high and low production capacity (Directive 92/5/EEC): thirty-six "*traditional*" and five "*smoked*" (horse-shoe shape, 30-35 mm∅, ripening of 10-25days), two "*cylindrical*" (30-35 mm∅, ripening of 10-15days), and twelve "*thin*" (horse-shoe shape, about 20 mm∅, ripening of less than 10 days). All the samples were analysed for the following parameters: a) *pH*, b) *aw* (Greco *et al.,* 2005), c) *microbiological parameters*, were determined using the A.P.H.A. methods (2001) or other specific protocols: Coliforms and *Escherichia coli*; *Pseudomonas* spp.; Mesophilic Anaerobic Sporeformers; Coagulase negative staphylococci (CNS); Lactic acid bacteria (LAB); Yeasts and Molds; *Clostridium perfringens*; *Salmonella* spp.; *Staphylococcus aureus*; *Listeria monocytogenes (L.m.),* (ISO 11290-1:1996 and ISO 11290-2:1998). Data were subjected to analysis of variance using the GLM procedures. The LSD test was used to evaluate differences between means (Statgraphics Plus, 5.1, Manugistics, MD, USA). Biochemical characterisation and presumptive identification were performed on all the *Listeria spp.* isolates, using the API Listeria identification system (Bio Mérieux). All the *Listeria monocytogenes* isolates were further characterised by a PCR-based method, aimed at *prfA* gene fragment detection (Jofré *et al.,* 2005) and by serotyping (Ueda *et al.,* 2002).

Results and Discussion

The pH, a_w (numeric values) and microbial ($\log_{10}$ CFU g^{-1}) results are presented in Table 1 and in the text as mean ± sd. a) *pH*: more than 90% of the sausages showed values ranging from 5.5 to 6, with no significant ($p>0.05$) differences between the sausage types. b) *aw*: the lower values were observed in the *"thin"* type sausage (0.87±0.01). More than 70% of the sausages showed values ranging from 0.90 to 0.92, while 20% ("*traditional*" type) showed values up to 0.94. c) *Microbiological parameters: Coliforms* were detected in 80% of samples with no significant differences in levels found between the sausage types. More than 80% of the positive samples, showed values ranging from 3 to 5 $\log_{10}$ CFU g^{-1}, and in the 15% of *"traditional"* sausages higher than 5 $\log_{10}$ CFU g^{-1}. *E.coli* was detected in 25.5% of samples tested. The highest contamination level ($p<0.05$) was found in samples with high pH values. *Pseudomonas spp.* was presence in all samples (4.58±0.87). In more than 30% of "*traditional*" and *"thin"* sausages, *Pseudomonas* spp. levels higher than 5 $\log_{10}$ CFU g^{-1} were detected. *Mesophilic Anaerobic Sporeformers* were detected in 10.9% of samples, and detected mainly in *"traditional"* (17.2%) and "*cylindrical"*(50%) sausage types. A significantly higher prevalence ($p<0.05$) of Mesophilic Anaerobic Sporeformers were found in sausages with a ripening period of less than 15 days (< 15days). *LAB* was the most prevalent microflora in all samples. Sausages with extended ripening period had the highest ($p<0.01$) levels of LAB. In 10% of "*traditional*" sausages, values higher than 8 $\log_{10}$ CFU g^{-1} LAB were found. *CNS* was found in more than 80% of samples, with some samples having values higher than 6 $\log_{10}$ CFU g^{-1}. *Yeasts and Molds* high levels of yeasts and moulds were found in all sample types. *Cl. Perfringens* was recovered from 14.5% of samples (all types), samples with high pH value were the most contaminated ($p<0.05$). *S. aureus* was detected in 14.5% of samples, and was strongly associated with lower ripening period ($p<0.05$) and high pH value ($p<0.01$). *Listeria spp and L isteria monocytogenes*, only twenty-one samples were analysed for *Listeria* spp. Of the 21 samples tested, *Listeria* spp. was detected in 76.1% of samples while *L. monocytogenes* was detected in 56.2% of samples. The

contamination rate was <100cfu/g for all samples. Fifty-nine strains were identified as belonging to the following species: forty-two were *L. innocua*, fourteen were *L. monocytogenes* and three were *L. welshimeri*. All the *L. monocytogenes* isolates showed the same electrophoretical pattern (amplification product of 464*bp*). Serotyping revealed that fourteen *L.monocytogenes* strains belonged to the following serotypes: 1/2a (57.1%), 4b (28.5%), 3b (7.1%), 1/2c (7.1%). Thevenot *et al.,* (2005) reported a high heterogeneity in serotypes collected in sausages. This may allow non specific serotype to survive sausage processing and maturation. *Salmonella* spp. were not found in any of the samples.

Table 1: Physicochemical and microbiological parameters (mean±s.d.) of the different types of "Salsiccia Sarda" at the end of ripening.

Type Parameters	Traditional 36[1]	Smoked 5[1]	Cylindrical 2[1]	Thin 12[1]	Total 55[1]
pH	5.54± 0.37	5.46±0.29	5.50±0.41	5.69±0.35	5.56±0.35
a_w	0.90± 0.02	0.92±0.03	0.92±0.00	0.89±0.02	0.90±0.02
Coliforms	3.47± 1.28 (28)[2]	3.60±0.23 (4)[2]	3.11± 1.37	3.13± 1.26 (10)[2]	3.39 ± 1.18
E.coli	2.51± 1.23 (3)[2]	2.78 (1)[2]	2.60 (1)[2]	2.57± 1.32 (3)[2]	2.55±1.09
Pseudomonas spp	4.70±0.64	4.42 ± 0.17	4.48±0.47	4.74± 0.61	4.58±0.87
Anaerobes sporeformers	1.73±0.83	-	4.48 (1)[2]	-	2.19 ± 1.34
LAB	7.27± 0.73	7.03±0.71	7.23±1.19	7.48±1.05	7.29±0.80
CNS	6.83± 0.79	6.78±0.22	6.18±1.21	6.71±0.47	6.77±0.70
Yeasts and Molds	5.07± 0.49	5.39±0.14	5.39±0.10	5.16±0.34	5.13±0.43
Cl.perfringens	2.34±0.62(4)[2]	1.30 (1)[2]	5.0±2.46 (1)[2]	1.0 (1)[2]	2.14 ± 1.20
S.aureus	5.82±0,70 (4)[2]	4.0 (1)[2]	5.0 (1)[2]	4.17 ± 0.56 (2)[2]	5.07±0.98
L. monocytogenes	7/14[3]	-	1/1[3]	1/2[3]	9/14[3]

[1]= number of samples; [2]= mean±s.d referred to the number of positives in bracket; [3]=number of positive samples; - =below the sensibility limit of the method

Conclusions

The results indicate an incorrect management of the acidification (more than 90% of the samples showed values of pH higher than 5.5) and drying steps (the a_w value was higher than 0.90 in more than 70% of samples) during processing. These processing conditions may be ineffective in pathogen and spoilage bacterial control. Although the low contamination rate (<100cfu/g), the presence of *L. monocytogenes* in fermented sausages at the time of consumption still remain a major concern for manufacturers (Thevenot *et al.,* 2005). To increase the safety of these products, efforts should be encouraged to achieve the effectiveness of the following hurdles: a_w reduction (extended ripening) and pH reduction (Barbuti and Parolari, 2002). Effective hygiene control strategies should also be ensured to prevent cross contamination of products.

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